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62. The composition of claim 60 or 61, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

63. (New) The method of claim 33, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

REMARKS

Upon entry of this amendment, claims 1-3, 6, 9-15, 19, 21-27, 31-63 are pending. No new matter is introduced by this amendment. Support for the newly added claims may be found throughout the specification and at least at pages 19-34 and pages 52-61.

CONCLUSION

Applicants submit that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: December 16, 2002

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Marked-up version showing claim amendment

In the Claims

Please cancel claims 4-5, 7-8, 16-18, 20, 28-30 and replace claims 1-3, 6, 9-15, 19, 21-27, 31-62 with the following claims.

1. (Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme [comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity]is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

2. The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

3. The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

4. (Cancelled) The enzyme mixture of claim 1, wherein said second enzyme is a mutant DNA polymerase.

5. (Cancelled) The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

6. (Amended) An enzyme mixture [for DNA synthesis]comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

7. (Cancelled) An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, said second enzyme is a

mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

8. (Cancelled) The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

9. (Amended) The enzyme mixture of claim 6, [7, or 8,]wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

10. (Amended) The enzyme mixture of claim 1 or 9, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

11. (Amended) The enzyme mixture of claim [6, 7, or 8]1, further comprising a PCR enhancing factor and/or an additive.

12. (Amended) The enzyme mixture of claim [8]6, wherein said mutant Pfu DNA polymerase comprises a mutation in its partitioning domain or the polymerase domain.

13. (Amended) A kit comprising a first enzyme, [and]a second enzyme, and packaging material therefor, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme [comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity, and packaging material therefore] is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

14. (Amended) The kit of claim [12]13, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

15. The kit of claim 14, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA

polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

16. (Cancelled) The kit of claim 15, wherein said second enzyme is a mutant DNA polymerase.

17. (Cancelled) The kit of claim 16, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

18. (Cancelled) The kit of claim 17, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

19. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

20. (Cancelled) A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a Taq DNA polymerase, and packaging material therefore, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

21. (Amended) The kit of claim [12,] 13 or 19,[or 20,] further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

22. (Amended) The kit of claim [16,]19, [or 20,]wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

23. (Amended) The kit of claim 13 or 22, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

24. (Amended) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme [comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity] which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

25. (Amended) The method of claim 24, wherein said nucleic acid template is a DNA [or an RNA] molecule.

26. The method of claim 25, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

27. The method of claim 26, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

28. (Cancelled) The method of claim 25, wherein said second enzyme is a mutant DNA polymerase.

29. (Cancelled) The method of claim 28, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

30. (Cancelled) The method of claim 28, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

31. A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

32. A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

33. (Amended) The method of claim [29,]31, or 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

34. (Amended) The method of claim 24 [33], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

35. The method of claim 24, 31 or 32, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

40. A mutant Pfu DNA polymerase with reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

41. The mutant DNA polymerase of claim 40, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

47. A composition comprising a mutant Pfu DNA polymerase, wherein said mutant DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

48. The composition of claim 47, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

54. A kit comprising a mutant DNA polymerase which comprises a reduced DNA polymerization activity and packaging material therefor, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

55. The kit of claim 54, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

57. A mutant Pfu DNA polymerase produced by introducing a mutation in to a polynucleotide encoding a wild type Pfu DNA polymerase to produce a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

58. A mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) providing a polynucleotide encoding a wild-type Pfu DNA polymerase;

(b) introducing one or more nucleotide mutations into said polynucleotide to produce a mutant polynucleotide encoding said mutant Pfu DNA polymerase; and

(c) expressing said mutant polynucleotide to produce said mutant Pfu DNA polymerase, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

59. The mutant DNA polymerase of claim 58, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

60. A composition comprising a mutant Pfu DNA polymerase produced by expressing a polynucleotide encoding a Pfu DNA polymerase with a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

61 A composition comprising a mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) introducing a mutation into a polynucleotide encoding a wild-type Pfu DNA polymerase to produce said mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388;

(c) expressing said mutant polynucleotide to produce said composition comprising said mutant Pfu DNA polymerase.

62. The composition of claim 60 or 61, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

63. (New) The method of claim 33, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.